

We claim:

1. A composition consisting essentially of a pharmaceutically acceptable excipient and a polynucleotide adsorbed to a cationic microparticle, wherein said polynucleotide comprises a coding sequence that encodes a hepatitis C virus (HCV) immunogen operably linked to control elements that direct the transcription and translation of said coding sequence *in vivo*, and further wherein the HCV immunogen is an immunogenic HCV E1E2 complex with a contiguous sequence of amino acids having at least 80% sequence identity to the contiguous sequence of amino acids depicted at positions 192-809 of Figures 2A-2C, with the proviso that said polynucleotide does not encode an HCV immunogen other than the HCV E1E2 complex.
2. The composition of claim 1, wherein said HCV E1E2 complex consists of the sequence of amino acids depicted at positions 192-809 of Figures 2A-2C.
- 15 3. The composition of claim 1, wherein the cationic microparticle is formed from a polymer selected from the group consisting of a poly(α -hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, and a polyanhydride.
- 20 4. The composition of claim 3, wherein the cationic microparticle is formed from a poly(α -hydroxy acid) selected from the group consisting of poly(L-lactide), poly(D,L-lactide) and poly(D,L-lactide-co-glycolide).
- 25 5. The composition of claim 4, wherein the cationic microparticle is formed from poly(D,L-lactide-co-glycolide).
6. A composition consisting essentially of:
 - (a) a pharmaceutically acceptable excipient; and
 - (b) a polynucleotide adsorbed to a cationic microparticle formed from poly(D,L-lactide-co-glycolide), wherein said polynucleotide comprises a coding sequence that encodes a hepatitis C virus (HCV) immunogen operably linked to

control elements that direct the transcription and translation of said coding sequence *in vivo*, and further wherein the HCV immunogen is an HCV E1E2 complex consisting of the sequence of amino acids depicted at positions 192-809 of Figures 2A-2C, with the proviso that said polynucleotide does not encode an HCV immunogen other than the HCV E1E2 complex.

7. A method of stimulating an immune response in a vertebrate subject which comprises administering to the subject a therapeutically effective amount of a first composition consisting essentially of a pharmaceutically acceptable excipient and a 10 polynucleotide adsorbed to a cationic microparticle, wherein said polynucleotide comprises a coding sequence that encodes a hepatitis C virus (HCV) immunogen operably linked to control elements that direct the transcription and translation of said coding sequence *in vivo*, and further wherein the HCV immunogen is an immunogenic HCV E1E2 complex with a contiguous sequence of amino acids having 15 at least 80% sequence identity to the contiguous sequence of amino acids depicted at positions 192-809 of Figures 2A-2C, with the proviso that said polynucleotide does not encode an HCV immunogen other than the HCV E1E2 complex, wherein said HCV E1E2 complex is expressed *in vivo* to elicit an immune response.

8. The method of claim 7, wherein said HCV E1E2 complex consists of the 20 sequence of amino acids depicted at positions 192-809 of Figures 2A-2C.

9. The method of claim 7, wherein the cationic microparticle is formed from a polymer selected from the group consisting of a poly(α -hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, and a poly-anhydride.

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10. The method of claim 9, wherein the cationic microparticle is formed from a poly(α -hydroxy acid) selected from the group consisting of poly(L-lactide), poly(D,L-lactide) and poly(D,L-lactide-co-glycolide).

11. The method of claim 10, wherein the cationic microparticle is formed from poly(D,L-lactide-co-glycolide).
12. The method of claim 7, further comprising administering a therapeutically effective amount of a second composition to the subject, wherein the second composition comprises an immunogenic HCV polypeptide and a pharmaceutically acceptable excipient.
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13. The method of claim 12, wherein said second composition is administered subsequent to the first composition.
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14. The method of claim 12, wherein said immunogenic HCV polypeptide in said second composition is an immunogenic HCV E1E2 complex with a contiguous sequence of amino acids having at least 80% sequence identity to the contiguous
15 sequence of amino acids depicted at positions 192-809 of Figures 2A-2C.
15. The method of claim 14, wherein said HCV E1E2 complex consists of the sequence of amino acids depicted at positions 192-809 of Figures 2A-2C.
16. The method of claim 12, wherein said second composition further
20 comprises an adjuvant.
17. The method of claim 16, wherein said adjuvant is a submicron oil-in-water emulsion capable of enhancing the immune response to the immunogenic HCV polypeptide, wherein the submicron oil-in-water emulsion comprises (i) a
25 metabolizable oil, wherein the oil is present in an amount of 1% to 12% of the total volume, and (ii) an emulsifying agent, wherein the emulsifying agent is present in an amount of 0.01% to 1% by weight (w/v) and comprises polyoxyethylene sorbitan mono-, di-, or triester and/or a sorbitan mono-, di-, or triester, wherein the oil and the emulsifying agent are present in the form of an oil-in-water emulsion having oil
30 droplets substantially all of which are about 100 nm to less than 1 micron in diameter.

18. The method of claim 17, wherein the submicron oil-in-water emulsion comprises 4-5% w/v squalene, 0.25-1.0% w/v polyoxyethylenesorbitan monooleate, and/or 0.25-1.0% sorbitan trioleate, and optionally,

5 N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(l'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

19. The method of claim 17, wherein the submicron oil-in-water emulsion consists essentially of about 5% by volume of squalene; and one or more emulsifying

10 agents selected from the group consisting of polyoxyethylenesorbitan monooleate and sorbitan trioleate, wherein the total amount of emulsifying agent(s) present is about 1% by weight (w/v).

20. The method of claim 19, wherein the one or more emulsifying agents are

15 polyoxyethylenesorbitan monooleate and sorbitan trioleate and the total amount of polyoxyethylenesorbitan monooleate and sorbitan trioleate present is about 1% by weight (w/v).

21. The method of claim 12, wherein said second composition further

20 comprises a CpG oligonucleotide.

22. A method of stimulating an immune response in a vertebrate subject which comprises:

(a) administering to the subject a therapeutically effective amount of a first

25 composition consisting essentially of a polynucleotide adsorbed to a cationic microparticle formed from poly(D,L-lactide-co-glycolide), wherein said polynucleotide comprises a coding sequence that encodes a hepatitis C virus (HCV) immunogen operably linked to control elements that direct the transcription and translation of said coding sequence *in vivo*, and further wherein the HCV immunogen

30 is an HCV E1E2 complex consisting of the sequence of amino acids depicted at positions 192-809 of Figures 2A-2C, with the proviso that said polynucleotide does

not encode an HCV immunogen other than the HCV E1E2 complex, and wherein said HCV E1E2 complex is expressed *in vivo*; and

(b) administering a therapeutically effective amount of a second composition to the subject, wherein the second composition comprises (i) an immunogenic HCV E1E2 complex consisting of the sequence of amino acids depicted at positions 192-809 of Figures 2A-2C, (ii) an adjuvant, and (iii) a pharmaceutically acceptable excipient, to elicit an immune response in the subject.

23. The method of claim 22, wherein said adjuvant is a submicron oil-in-water emulsion capable of enhancing the immune response to the immunogenic HCV E1E2 complex in the second composition, wherein the submicron oil-in-water emulsion comprises (i) a metabolizable oil, wherein the oil is present in an amount of 1% to 12% of the total volume, and (ii) an emulsifying agent, wherein the emulsifying agent is present in an amount of 0.01% to 1% by weight (w/v) and comprises polyoxyethylene sorbitan mono-, di-, or triester and/or a sorbitan mono-, di-, or triester, wherein the oil and the emulsifying agent are present in the form of an oil-in-water emulsion having oil droplets substantially all of which are about 100 nm to less than 1 micron in diameter.

24. The method of claim 23, wherein the submicron oil-in-water emulsion comprises 4-5% w/v squalene, 0.25-1.0% w/v polyoxyelthylenesorbitan monooleate, and/or 0.25-1.0% sorbitan trioleate, and optionally, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(Γ -2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

25. The method of claim 23, wherein the submicron oil-in-water emulsion consists essentially of about 5% by volume of squalene; and one or more emulsifying agents selected from the group consisting of polyoxyelthylenesorbitan monooleate and sorbitan trioleate, wherein the total amount of emulsifying agent(s) present is about 1% by weight (w/v).

26. The method of claim 25, wherein the one or more emulsifying agents are polyoxyethylenesorbitan monooleate and sorbitan trioleate and the total amount of polyoxyethylenesorbitan monooleate and sorbitan trioleate present is about 1% by weight (w/v).

5 27. The method of claim 23, wherein said second composition further comprises a CpG oligonucleotide.

28. A method of making a composition comprising combining a pharmaceutically acceptable excipient with a polynucleotide adsorbed to a cationic
10 microparticle, wherein said polynucleotide comprises a coding sequence that encodes a hepatitis C virus (HCV) immunogen operably linked to control elements that direct the transcription and translation of said coding sequence *in vivo*, and further wherein the HCV immunogen is an immunogenic HCV E1E2 complex with a contiguous sequence of amino acids having at least 80% sequence identity to the contiguous
15 sequence of amino acids depicted at positions 192-809 of Figures 2A-2C, with the proviso that said polynucleotide does not encode an HCV immunogen other than the HCV E1E2 complex.

29. Use of a composition according to any of claims 1-6 in a method for
20 stimulating an immune response in a vertebrate subject.